Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

FINAL REPORT

Final reports will be published on the Cal Poly Digital Commons website(http://digitalcommons.calpoly.edu).

I. Project Title
Engineering Injectable, Self-Assembling 3D Vasculature

II. Project Completion Date
All data collection was completed in July 2018 and analyses carried out by January 2019.

III. Student(s), Department(s), and Major(s)

(1) Kendyl Cohn, College of Engineering, BMS General and Biomedical Engineering

IV. Faculty Advisor and Department
Dr. Christopher Heylman, Biomedical Engineering

V. Cooperating Industry, Agency, Non-Profit, or University Organization(s)
Biomedical Engineering Department, College of Engineering

VI. Executive Summary

The Heylman Lab is developing strategies for engineering tissue-on-a-chip models for drug screening and drug-delivery testing. To create these tissues-on-a-chip, tissue components (cells, ECM proteins, growth factors) are seeded into a microfluidic device and cultured in a biophysical environment the mimics in vivo conditions to form 3D tissue structures. The scope of this project was to establish the necessary combination of cell types, extracellular matrix proteins, and nutrients to enable the self-assembly of 3D vessel networks in vitro; another project was performed in parallel to develop an appropriate microfluidic device. Additionally, a MatLab model was developed in this project to demonstrate increased nutrient delivery through tissue with capillaries compared to non-

To create capillary networks in vitro, three main components are required: endothelial cells (ECs) to make capillaries, fibroblasts to secrete angiogenic signals, and extracellular matrix (ECM) proteins to provide structure. To co-culture these different cell types together to make 3D capillary networks, the following were determined through individual experiments: ideal culture medium and time, optimal density of each cell type, and a successful differential staining protocol. Human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs) were combined in a fibrinogen solution (clot precursor), injected into culture wells and gelled into fibrin by adding the clotting enzyme thrombin. The cells, suspended throughout the 3D space, were then cultured in endothelial growth medium with vascular endothelial growth factor for 9 days prior to fluorescent immunostaining and imaging with laser scanning confocal microscopy. The results revealed the formation of an interconnected 3D capillary network spanning the 0.25mm thick, 1cm^2 area. The

protocols developed by this project to create this injectable, self-assembling vasculature using easy to obtain and culture cell types can now be used to develop tissue-on-a-chip models.

VII. Major Accomplishments

(1) Optimized 3D capillary network preparation protocol: culture media formulation, endothelial to fibroblast cell density, and culture conditions with differential identification of cell types

(2) Three-dimensional capillary networks embedded in a scaffold of extracellular matrix proteins capable of convective mass transport (nutrient provision/waste removal) with quantitative and qualitative assessment of vessels.

(3) Comparison of mass transport in tissue with and without engineered capillary network using an expandable mathematical model.

VIII. Expenditure of Funds

Reagents and Materials	*qty*	*price/unit*
Human Umbilical Vein Endothelial Cells	1 vial	$199.00
Human Dermal Fibroblasts	1 vial	$239.00
Endothelial Cell Growth Kit – VEGF	3 kits	$139.00
Fibroblast Growth Kit, Low Serum	1 kit	$83.00
Dulbecco's Modified Eagle Medium (DMEM)	1 bottle	$11.05
Phenol Red	1 vial	$26.50
Dulbecco's Phosphate Buffered Saline	1 bottle	$46.20
Trypsin/EDTA solution	1 bottle	$41.40
Trypan Blue Solution	1 bottle	$41.40
Trypsin Neutralizing Solution	1 bottle	$29.80
Fetal Bovine Serum	1 bottle	$150.00
Penicillin/Streptomycin Solution	1 bottle	$6.43
Amphotericin B, Fungizone	1 bottle	$14.16
70% Isopropyl Alcohol	1 bottle	$20.66
15 mL centrifuge sterile conicals	1 case	$65.12
50 mL centrifuge sterile conicals	1 case	$87.46
2 mL aspirating sterile pipettes	1 case	$22.44
5 mL graduated sterile pipettes	1 case	$21.26
10 mL graduated sterile pipettes	1 case	$22.53
25 mL graduated sterile pipettes	1 case	$62.22
Small nitrile gloves	1 pack	$4.65
T75 sterile culture flasks	1 case	$60.85
Sterile 1000 microliter micropipette tips	1 pack	$40.99
Sterile 100 microliter micropipette tips	1 pack	$48.50
Mr. Frosty – 18 cryovial capacity	1 container	$57.57

Nunc Cryovials	1 pack	$122.27
100% IPA for cryopreservation	1 case	$71.63
Dimethyl Sulfoxide (DMSO)	1 bottle	$117.00
Hoechst 33342 nucleotide stain	1 bottle	$56.20
PECAM-1 Antobidy (H-3)	1 vial	$314.00
	Total	*$2,500.29*
	Amount Awarded	**$2,500.00**

IX. Impact on Student Learning

At the start of this project, the goal of creating a self-assembling *in vitro* capillary network seemed lofty and nearly unattainable given the relatively limited resources of a new research lab. However, thanks to the Baker/Koob Endowments, I was able to push through the pitfalls inherent in life-based research and successfully achieve the objectives laid out at the start of the project. Support from Baker/Koob not only provided the means for me to carry out such resource-consuming research, but also gave allowed me to expand my work to include the use of different cell types and optimize techniques for visualizing vascular networks with fluorescent microscopy. This year-long research project helped me apply my academic knowledge to research, strengthen laboratory and MatLab skills, and solidify my career aspirations.

Prior to this work, I had cursory knowledge of mathematical models for biological processes and limited experience with tissue engineering-based research. Through this project, I taught myself basic biotransport phenomena and MatLab coding in order to develop a mathematical model of glucose transport through living tissues. This was immensely valuable for learning to self-teach complex topics and greatly expanded my skill set as an engineer. Additionally, as is inherent in tissue engineering, I got the opportunity to directly apply concepts from many physiology, chemistry, and biology courses to the engineering process. The multidisciplinary nature of this project forced me to think outside the boxes of ‘scientist’ and ‘engineer’ and to combine these disciplines for a deeper understanding that led to successful results.

Ultimately, this project culminated in a thesis, augmented by the aid of this award, that enabled me to complete my Master’s degree. Not only did this facilitate achieving my academic goals; the success of this research project had a huge impact on my career goals. This project was pivotal for getting hired as an engineer for a medical device and global health company, Penumbra, Inc., and in the determination of my aspirations to pursue a PhD in tissue engineering. I am grateful to the Cal Poly community for enabling me to ‘Learn by Doing’ and setting me up for success.